PATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
440131/PALL		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 22478	29/09/1999	02/10/1998
Applicant		
PALL CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/22478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61M1/36 B01D69/12 B01D61/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61M B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 17894 A (TRAVENOL LAB ISRAEL LTD ;KRAUS MENACHEM (IL); YONATH JACOB (IL)) 18 August 1994 (1994-08-18) page 4, line 10 -page 5, line 14 ---	1-16
A	WO 98 19722 A (PALL CORP ;BAXTER INT (US)) 14 May 1998 (1998-05-14) claim 9 ---	1-16
A	US 5 501 795 A (GSELL THOMAS C ET AL) 26 March 1996 (1996-03-26) claims 1,2 ---	1-16
A	EP 0 333 119 A (TERUMO CORP) 20 September 1989 (1989-09-20) abstract --- -/--	1-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2000	08/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Faria, C

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/22478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 783 094 A (GAMLIELI YEPHET ET AL) 21 July 1998 (1998-07-21) column 4, line 41 - line 49	1-16

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/22478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9417894 A	18-08-1994	IL 104670 A	05-04-1998
		EP 0683687 A	29-11-1995
WO 9819722 A	14-05-1998	AU 5005997 A	29-05-1998
		EP 0938351 A	01-09-1999
		NO 992162 A	06-07-1999
US 5501795 A	26-03-1996	US 5344561 A	06-09-1994
		US 5229012 A	20-07-1993
		AT 98880 T	15-01-1994
		CA 2016297 A,C	09-11-1990
		DE 69005354 D	03-02-1994
		DE 69005354 T	19-05-1994
		EP 0397403 A	14-11-1990
		ES 2047851 T	01-03-1994
		GB 2231282 A,B	14-11-1990
		JP 2541340 B	09-10-1996
		JP 3027317 A	05-02-1991
EP 0333119 A	20-09-1989	JP 1232973 A	18-09-1989
		JP 1888119 C	07-12-1994
		JP 6014969 B	02-03-1994
		AU 608133 B	21-03-1991
		AU 3130889 A	23-11-1989
		DE 68911176 D	20-01-1994
		DE 68911176 T	28-04-1994
		KR 9108638 B	19-10-1991
		US 4963260 A	16-10-1990
US 5783094 A	21-07-1998	US 5895575 A	20-04-1999
		AU 5309396 A	30-10-1996
		EP 0869835 A	14-10-1998
		WO 9632178 A	17-10-1996

PATENT COOPERATION TREATY

RECD 05 JAN 2001

WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

15

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
440131/PALL		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/22478	29/09/1999	02/10/1998
International Patent Classification (IPC) or national classification and IPC A61M1/36		
Applicant PALL CORPORATION et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25/04/2000	02.01.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Péru, L Telephone No. +49 89 2399 2377

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US99/22478

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-17 as originally filed

Claims, No.:

1-16 as originally filed

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9, 11, 15-16
	No:	Claims	10, 12-14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9, 11, 15-16
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

V. Novelty and inventive step

Reference is made to the following documents:

D1 . . . WO 98/19722

D2 . . . US-A-5 501 795

1 Document D1 discloses (claim 9) a filter device for processing a biological fluid comprising a housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet, a filter disposed in the housing across the fluid flow path, the filter comprising a first filter element (intermediate membrane) comprising a porous fibrous leukocyte depletion medium and a second filter element (final membrane) comprising a porous membrane having a pore size of about 5 micrometers or less and disposed downstream of the first filter element, wherein the filter is arranged to allow plasma to pass there through and substantially prevent the passage of leukocytes there through.

The only difference between the device disclosed in D1 and the subject-matter of claim 1 concerns the surface tension of the first filter element, which is not mentioned in D1.
First, it is not clear whether this parameter could be a novelty criterion (Guidelines III-4.7a), especially since the description itself presents the surface treatment as an example (page 8 line 1).
Second, the possible treatment is, as also disclosed in the description, already well-known for increase in efficiency. It has e.g. being extensively disclosed in document D2 (column 8 line 44 - column 9 line 35) for a leukocyte filter. Its inclusion in the device of D1 would be matter of normal design possibility.
Third, the value 70 itself is described in document D2.

The subject-matter of claim 1, if novel, thus appears not to involve an inventive step (Article 33.3 PCT).

2 Dependent claims 2-9 (see item VIII.1 for claim 2) do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.

- claims 2-3: The inclusion of a red blood cell barrier in the first filter is matter of normal possibilities depending on the intended result: if the red cells cause problem, it is obvious to decide to remove them, and the inclusion of the barrier with the leukocyte depletion medium is the obvious possibility since combined filters are known (see the documents cited in the present description page 7 lines 32-34).
- claims 4, 5, 7, 8: The choice of such commercial first filter elements comes within the common practice (see the description or document WO 94/17894).
- claim 6: The value of CWST does not bring any unforeseen advantages and is a simple choice within an available range.
- claim 9: The normal design of systems for processing blood is the insertion of the filter device between two containers.

3 Document D1 discloses a method for processing a biological fluid comprising passing the fluid through a filter device comprising a filter including a fibrous leukocyte depletion medium and a membrane, and collecting the filtered fluid. This fluid has been subjected to centrifugation for obtaining the supernatant (page 3 line 9).
The subject-matter of claims 10, 12-14 thus is not novel (Article 33.2 PCT).

4 The method of claim 11 (see however item VIII.5) for a similar reason as in item V.2 is not novel or at least does not involve an inventive step (Article 33.3 PCT).

5 The methods of claims 15 and 16 (see item VIII.6) directly derive from the device of claim 1 and as such do not involve an inventive step neither.

VII. Other remarks

1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in e.g. the document D1 is not mentioned in the background of the invention described, nor is this document identified therein.

2 The features of the claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

3 The signification of "CWST" should have been specified at its first occurrence on page 3.

4 The units "dynes", "inch" employed in claims 1, 2, 6 and on pages 3, 8, 13, 16 are not additionally expressed in terms of the units stipulated by Rule 10.1(a) PCT.

5 The mention "incorporated by reference" (pages 1 and 17) is not accepted by all Examination Authorities and should have been deleted (Guidelines PCT, II-4.17). Moreover, the first paragraph should have been deleted since it is unclear what it means, and the two last paragraphs should have been deleted since they appear to be superfluous (Guidelines PCT, III-4.3a).

VIII. Clarity

1 Although claims 1 and 2 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought: Claim 2 is identical to claim 3 and should have depended on claim 1.

Same can be said for independent claims 10, 11, 12, 14, 15 which all relate to a method for processing a biological fluid.

The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection.
Hence, the claims do not meet the requirements of Article 6 PCT.

2 Some of the features in the apparatus claims 1 and 2 relate to a method of using the apparatus rather than clearly defining the apparatus in terms of its technical features: "the filter is arranged to ... there through". The intended limitations are therefore not clear from this claim, contrary to the requirements of Article 6 PCT.

3 The term "CWST" used in claims 1, 2, 6 is unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

4 Claim 8 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claim attempts to define the subject-matter only in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result should be added.

5 The last step of the method defined in claim 11 is unclear since it is supposed to collect a fluid substantially free of leukocytes whereas the fluid is passed through a fibrous red blood cell barrier medium.

6 Claim 16 is unclear since it relates to the step of collecting a filter plasma-rich biological fluid which is substantially free of leukocytes and/or red blood cells (according to the claims on which claim 16 depends), which collection should comprise the step of passing a leukocyte-containing fluid through a filter.

7 The mention to "the spirit of the invention" on page 17 implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity (Article 6 PCT) when used to interpret them (Guidelines PCT, III-4.3a).

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61M 1/36, B01D 69/12, 61/18

A1

(11) International Publication Number: WO 00/20053

(43) International Publication Date: 13 April 2000 (13.04.00)

(21) International Application Number: PCT/US99/22478

(22) International Filing Date: 29 September 1999 (29.09.99)

(30) Priority Data:
60/102,973 2 October 1998 (02.10.98) US

(71) Applicant *(for all designated States except US)*: PALL CORPORATION [US/US]; 2200 Northern Boulevard, East Hills, NY 11548–1209 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BORMANN, Thomas, J. [US/US]; 29 Cawfield Lane, Melville, NY 11747 (US). DELGIACCO, Gerard, R. [US/US]; 196 Tibbets Road, Yonkers, NY 10705 (US). SELMAN, Byron [US/US]; 89 Abbott Drive, Huntington, NY 11743 (US).

(74) Agent: ZHU, Song; Leydig, Voit & Mayer, Ltd., 700 Thirteenth Street, N.W., Suite 300, Washington, DC 20005 (US).

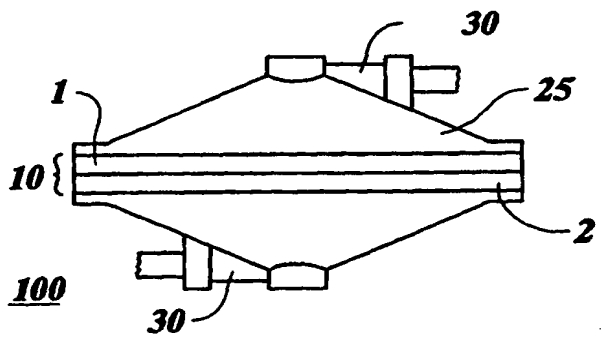
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: BIOLOGICAL FLUID FILTER AND SYSTEM

(57) Abstract

A filter for producing a plasma–rich fluid that is substantially free of leukocytes is disclosed.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Inter. ...onal Application No
PCT/US 99/22478

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61M1/36 B01D69/12 B01D61/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61M B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 17894 A (TRAVENOL LAB ISRAEL LTD ;KRAUS MENACHEM (IL); YONATH JACOB (IL)) 18 August 1994 (1994-08-18) page 4, line 10 -page 5, line 14	1-16
A	WO 98 19722 A (PALL CORP ;BAXTER INT (US)) 14 May 1998 (1998-05-14) claim 9	1-16
A	US 5 501 795 A (GSELL THOMAS C ET AL) 26 March 1996 (1996-03-26) claims 1,2	1-16
A	EP 0 333 119 A (TERUMO CORP) 20 September 1989 (1989-09-20) abstract	1-16

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2000	08/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Faria, C

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 99/22478

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 783 094 A (GAMLIELI YEPHET ET AL) 21 July 1998 (1998-07-21) column 4, line 41 - line 49	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]nal Application No
PCT/US 99/22478

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9417894 A	18-08-1994	IL 104670 A	05-04-1998
		EP 0683687 A	29-11-1995
WO 9819722 A	14-05-1998	AU 5005997 A	29-05-1998
		EP 0938351 A	01-09-1999
		NO 992162 A	06-07-1999
US 5501795 A	26-03-1996	US 5344561 A	06-09-1994
		US 5229012 A	20-07-1993
		AT 98880 T	15-01-1994
		CA 2016297 A,C	09-11-1990
		DE 69005354 D	03-02-1994
		DE 69005354 T	19-05-1994
		EP 0397403 A	14-11-1990
		ES 2047851 T	01-03-1994
		GB 2231282 A,B	14-11-1990
		JP 2541340 B	09-10-1996
		JP 3027317 A	05-02-1991
EP 0333119 A	20-09-1989	JP 1232973 A	18-09-1989
		JP 1888119 C	07-12-1994
		JP 6014969 B	02-03-1994
		AU 608133 B	21-03-1991
		AU 3130889 A	23-11-1989
		DE 68911176 D	20-01-1994
		DE 68911176 T	28-04-1994
		KR 9108638 B	19-10-1991
		US 4963260 A	16-10-1990
US 5783094 A	21-07-1998	US 5895575 A	20-04-1999
		AU 5309396 A	30-10-1996
		EP 0869835 A	14-10-1998
		WO 9632178 A	17-10-1996

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/545, B01D 67/00

A1

(11) International Publication Number: WO 98/21588

(43) International Publication Date: 22 May 1998 (22.05.98)

(21) International Application Number: PCT/US97/20475

(22) International Filing Date: 7 November 1997 (07.11.97)

(30) Priority Data:
08/748,711 14 November 1996 (14.11.96) US

(71) Applicant: PALL CORPORATION [US/US]; 2200 Northern Boulevard, East Hills, NY 11548 (US).

(72) Inventors: GELMAN, Charles; 2685 Hawthorne Road, Ann Arbor, MI 48104 (US). KONSTANTIN, Peter; 927 Coronado Drive, Gulf Breeze, FL 32561 (US). WIXWAT, Wilfrid, Klaus; 7592 Northpointe Boulevard, Pensacola, FL 32514 (US). YANG, Yujing; 7791 Northpointe Boulevard, Pensacola, FL 32514 (US). HOU, Chung-Jen; 2258 Oxford Drive, Pensacola, FL 32503 (US).

(74) Agents: KILYK, John, Jr. et al.; Leydig, Voit & Mayer, Ltd., Suite 4900, Two Prudential Plaza, Chicago, IL 60601-6708 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: MEMBRANE AND METHODS OF PREPARING AND USING SAME

(57) Abstract

The present invention provides a membrane comprising (a) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (b) a cellulose compound which allows for the detection of a biological molecule of interest, wherein the cellulose compound is uniformly distributed throughout the surface of the membrane. The present invention also provides methods of preparing and using such membranes.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

MEMBRANE AND METHODS OF PREPARING AND USING SAME

TECHNICAL FIELD OF THE INVENTION

The present invention relates to a membrane suitable for use in immunodiagnostic assays and blotting assays and methods of preparing and using same.

BACKGROUND OF THE INVENTION

Membranes have become invaluable tools in both the clinical and experimental biotechnological arts. Specifically, membranes are integral to immunodiagnostic assays and a variety of blotting assays. However, currently available membranes possess qualities which limit their utility within the context of the foregoing applications.

Immunodiagnostic assays are generally performed by applying a test liquid containing antigens to a porous membrane containing antibodies. As the test liquid laterally diffuses through the membrane, antibodies will bind antigens to which they are directed with a high degree of specificity. The binding of the antibodies to the antigens serves as a detection means (e.g., the visualization of the presence of antigens), and the specificity with which antibodies bind to antigens allows for the determination of whether or not the test liquid contains specific antigens. Therefore, in

immunodiagnostic assays, the membrane desirably possesses optimal immunodiagnostic properties. In other words, it is desirable that the membrane allow for optimal lateral diffusion of the test liquid, allow for adequate visualization of the existence of antigens in the test liquid (i.e., the membrane is capable of a high signal-to-noise ratio), allow for adequate protein binding, is hydrophilic, is capable of being uniformly manufactured in order to yield consistent results, and is safe to use.

Similarly, in a blotting assay, a membrane is contacted with a fluid comprising biological molecules such that the biological molecules become fixed to the membrane. Biological molecules of interest are subsequently visualized. It is desirable that the membrane utilized within the context of blotting assays have optimal blotting properties. Specifically, it is desirable that the membrane allow for the adequate binding of biological molecules, allow for adequate visualization of the biological molecules of interest (i.e., the membrane is capable of a high signal-to-noise ratio), is hydrophilic, is capable of being uniformly manufactured in order to yield consistent results, and is safe to use. However, unlike those membranes used in immunodiagnostic assays, blotting membranes need not allow for the lateral diffusion of biological molecules. In fact, for most blotting applications (e.g., southern blots, northern blots, western blots, and in situ hybridization of bacterial colonies), lateral diffusion is undesirable.

The most common types of membranes available for use in immunodiagnostic and blotting assays include

polyvinylidenefluoride, nylon, and cellulose-based membranes (e.g., nitrocellulose and cellulose acetate membranes). Each of the membranes, however, possesses qualities which limit its utility in the foregoing applications. Nitrocellulose is prepared by the nitration of naturally occurring cellulose. During nitration, a broad distribution of heterogenous oligomeric and polymeric nitrated products is produced as a consequence of the partial acid digestion of cellulose. Exacerbating the problem is the fact that the purity of the cellulose starting material depends on its source and pre-nitration treatment. As a result, uniformity in the manufacture of nitrocellulose membranes is difficult to achieve. For similar reasons, it is also difficult to achieve uniformity in the manufacture of other cellulosic membranes, such as cellulose acetate membranes. Furthermore, nitrocellulose membranes present numerous laboratory safety concerns by virtue of their flammability and explosiveness. Cellulose acetate and nitrocellulose membranes are also disadvantageous in that such membranes are very brittle, easily broken, and difficult to wet.

Nylon and polyvinylidenefluoride membranes also have disadvantages associated with their use within the context of the foregoing applications. Nylon membranes strongly bind biological molecules and, consequently, have low signal-to-noise ratios. Polyvinylidenefluoride and other synthetic polymeric membranes cannot be used in applications where surface activity, which facilitates the binding of biological molecules, is necessary or where high lateral flow rates are necessary.

In view of the foregoing problems, there exists a need for membranes which can be used more effectively in immunodiagnostic and blotting assays. The present invention provides such a membrane and methods for the preparation thereof. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

The present invention provides a membrane comprising (a) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (b) a cellulose compound which allows for the detection of a biological molecule of interest, wherein the cellulose compound is uniformly distributed throughout the surface of the membrane.

The present invention also provides methods of preparing such membranes. Specifically provided is a method of preparing a membrane comprising (a) selecting a base membrane comprising polymer solids, the polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin, and (b) uniformly coating the base membrane with a coating solution, the coating solution comprising a cellulose compound which allows for the detection of a biological molecule of interest and a cellulose dissolving agent which is a solvent for the cellulose compound and is a non-solvent for the base membrane, to provide a membrane wherein the cellulose

compound is uniformly distributed throughout the surface of the membrane. Also provided is a method of preparing a membrane comprising (a) admixing (i) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (ii) a cellulose compound, and (b) casting the admixture of step (a) to prepare a membrane wherein the cellulose compound is uniformly distributed throughout the surface of the membrane.

The present invention also provides a method of using a membrane to detect a biological molecule of interest comprising (a) contacting the membrane with a fluid comprising the biological molecule of interest and (b) detecting the biological molecule of interest on the membrane. Further provided is an immunodiagnostic assay kit comprising a membrane and a means for detecting a biological molecule of interest. Also provided is a blotting assay kit comprising a membrane and a blotting solution.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention may best be understood with reference to the following detailed description of the preferred embodiments. The present invention provides a membrane as well as methods for the preparation thereof. The present inventive membrane comprises (a) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (b) a cellulose compound which allows for the detection of a biological molecule of

interest, wherein the cellulose compound is uniformly distributed throughout the surface of the membrane.

The first present inventive method of preparing a membrane comprises (a) selecting a base membrane comprising polymer solids, the polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin, and (b) uniformly coating the base membrane with a coating solution, the coating solution comprising a cellulose compound which allows for the detection of a biological molecule of interest and a cellulose dissolving agent which is a solvent for the cellulose compound and is a non-solvent for the base membrane, to provide a membrane wherein the cellulose compound is uniformly distributed throughout the surface of the membrane. The second present inventive method of preparing a membrane comprises (a) admixing (i) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (ii) a cellulose compound, and (b) casting the admixture of step (a) to prepare a membrane wherein the cellulose compound is uniformly distributed throughout the surface of the membrane.

Polymer Solids

The polymer solids comprise a fully synthetic organic polymer and optionally a polymer of natural origin. The polymer solids preferably comprise 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin; more preferably, 80 wt.% or more of a fully

synthetic organic polymer and about 20 wt.% or less of a polymer of natural origin; even more preferably, 90 wt.% or more of a fully synthetic organic polymer and about 10 wt.% or less of a polymer of natural origin; and most preferably, 100 wt.% of a fully synthetic organic polymer (i.e., with no or substantially no polymer of natural origin).

The use of fully synthetic organic polymers within the context of the present invention provides an advantage over existing membranes consisting only of polymers of cellulose compounds and their derivatives. The use of fully synthetic organic polymers allows for enhanced uniformity in the preparation of the membranes, yielding more uniform experimental results, and reduces the hazards associated with membranes consisting only of polymers of cellulose compounds and their derivatives. While any suitable fully synthetic organic polymer can be used within the context of the present invention, preferred fully synthetic organic polymers include polyethersulfones; polysulfones; polyamides, including polyarylamides (aramides); polyetheramides; polyacetals; polyacrylonitrile and acrylonitrile copolymers such as poly(styrene/acrylonitrile); polyarylenesulfides; polyetherimides; polyetherketones, polyetheretherketones, and polyarylene(ether)ketone variants; polyimides; polyesters; polycarbonates; polyacrylates, including polymethacrylates, polyalkylacrylates, and the like; polystyrene; polyolefin homopolymers and copolymers such as polyethylene, polypropylene, polybutylene, and the like; halogenated polyolefins such as polyvinylchloride, polyvinylidenefluoride, polytetrafluoroethylene, and the

like; thermoplastic polyurethanes; and combinations thereof. More preferably, the fully synthetic organic polymer is a polyethersulfone, polysulfone, polyamide, polyolefin, polyimide, halogenated polyolefin, or a combination thereof.

"Synthetic" polymers which are derived from chemical modification of naturally occurring substances are not "fully synthetic polymers" as that term is defined herein. Examples of such polymers of natural origin include nitrocellulose, cellulose acetate, higher acetylated cellulose products such as cellulose triacetate, cellulose propionate, cellulose butyrate, cellulose xanthate, and the like, as well as combinations thereof.

Biological Molecule

As used herein, the term "biological molecule" includes any peptide, protein, nucleic acid, derivative thereof, or combination thereof. While the biological molecule of interest can be any peptide, protein, nucleic acid, derivative thereof, or combination thereof, the biological molecule of interest is preferably a protein, nucleic acid, or a protein-nucleic acid fusion molecule; more preferably, the biological molecule of interest is a protein, DNA, or RNA.

Cellulose Compound

The cellulose compound utilized within the context of the present invention serves many functions. As previously stated, in order for a membrane to be used effectively in an immunodiagnostic or a blotting assay,

the membrane's immunodiagnostic or blotting properties desirably are optimized. The membrane's signal-to-noise ratio desirably is such that the signal being emitted by the means for detecting the biological molecule of interest can be adequately detected, e.g., visualized. To that end, the cellulose compound can serve to reduce the binding of biological molecules not of interest while maintaining an acceptable level of binding of the biological molecule of interest. Such can be accomplished by exploiting the different binding constants that biological molecules have with respect to various cellulose compounds. For example, the use of cellulose compounds to reduce membrane adsorbability of proteins is well known in the art. *See, generally,* U.S. Patent 4,968,533 (Gsell). Furthermore, it is well known in the art that membranes which exhibit reduced protein adsorbability are even less able to adsorb nucleic acids. Also, the cellulose compound can serve to enhance the growth of microorganisms on the membrane when such a capability is desired. *See, generally,* U.S. Patent 5,595,893 (Pometto, III et al.).

While any suitable cellulose compound, and derivative thereof, can be used within the context of the present invention, preferred cellulose compounds include nitrocellulose, ether derivatives of cellulose, ester derivatives of cellulose, xanthate derivatives of cellulose, and combinations thereof. Preferably, the ether derivative of cellulose is methylcellulose, carboxylated alkyl cellulose, or hydroxyalkylcellulose. A preferred hydroxyalkylcellulose is hydroxypropylcellulose. The preferred ester derivative

of cellulose is cellulose acetate, and, preferably, the xanthate derivative of cellulose is rayon, cellophane, or viscose. More preferably, the cellulose compound is a blend of nitrocellulose and a cellulose derivative selected from the group consisting of an ether derivative of cellulose, an ester derivative of cellulose, a xanthate derivative of cellulose, and combinations thereof.

When nitrocellulose is used as either the polymer of natural origin and/or the cellulose compound, either alone or in combination with other polymers of natural origin and/or cellulose compounds, the nitrocellulose desirably is highly purified and, preferably, has a degree of nitration of from about 5% to about 14%; more preferably, the degree of nitration is from about 8% to about 12%; even more preferably, the degree of nitration is from about 11% to about 12%; and most preferably, the degree of nitration is from 11.3% to 11.8%. Furthermore, the nitrocellulose preferably has a viscosity of about 18 to about 45 cps; more preferably, the nitrocellulose has a viscosity of about 30 to about 35 cps, as measured by standard methods in a combined ethanol, toluene, ethyl acetate solvent. Lower viscosity nitrocellulose appears to provide superior membranes; however, nitrocellulose in the 30-35 cps range shows little difference over less viscous nitrocellulose and is more readily available.

It is desirable to minimize the amount of cellulose compound incorporated into the present inventive membrane in order to minimize the hazards associated therewith, but such a consideration must be viewed in light of the desire to produce a membrane with desirable

immunodiagnostic and/or blotting properties, which necessarily requires the use of a cellulose compound. Thus, the cellulose compound is preferably incorporated into the membrane in a quantity minimally required to realize desirable immunodiagnostic and/or blotting properties. In other words, the cellulose compound is preferably incorporated into the membrane in a quantity which is minimally required to realize an adequate signal-to-noise ratio (e.g., up to 1 wt.% based on the overall weight of the membrane). Therefore, for example, the constituents of a membrane can constitute 1 wt.% of a cellulose compound (e.g., nitrocellulose) and 99 wt.% of a 90:10 mixture of a fully synthetic organic polymer (e.g., polyethersulfone) and a polymer of natural origin (e.g., cellulose triacetate), respectively. Alternatively, the membrane can constitute up to 2 wt.%, up to 5 wt.%, or up to 10 wt.%, or even more, cellulose compound based on the weight of the membrane.

Not only is the quantity of the cellulose compound a significant consideration, but so too is the way in which the cellulose compound is distributed in the membrane. While the cellulose compound can be distributed throughout the surface of the membrane in any suitable way, preferably, it is uniformly or substantially uniformly distributed throughout the surface of the membrane. Uniform distribution is important because if the cellulose compound is non-uniformly distributed throughout the membrane, the localized concentration of the cellulose compound will vary throughout the surface of the membrane, and, consequently, the immunodiagnostic and/or blotting properties of the membrane will be non-

uniform, thereby adversely affecting performance. The distribution of the cellulose compound throughout the surface of the membrane can be such that the cellulose compound completely or substantially covers the entire surface of the membrane.

The cellulose compound can be a coating on the surface of the membrane, applied ex situ to an already formed membrane comprising the polymer solids. The cellulose compound also can be in admixture (i.e., form a blend) with the polymer solids from which a membrane comprising the admixture is formed by otherwise conventional methods (i.e., the cellulose compound is distributed in situ). Alternatively, the cellulose compound can be both a coating on the membrane and in admixture with the polymer solids forming the membrane. Also, the cellulose compound should be distributed in a quantity sufficient and in such a way so as to cause the cellulose compound to reside throughout the surface of the membrane, both on external surfaces and, if the membrane is porous, internal surfaces.

Membrane

The present inventive membrane can be either porous or non-porous. Whether the present inventive membrane is porous or non-porous is dictated by the context in which the membrane is to be used, e.g., by the sensitivity of the means used to detect the biological molecule of interest. For blotting assays which utilize more sensitive detection means, the membrane need not be porous because the biological molecule of interest can be fixed at the surface of the membrane in a quantity

sufficient to allow detection. For blotting assays which utilize less sensitive detection means, it is generally preferred that the membrane be porous to allow for a larger quantity of biological molecules to be fixed to the membrane and consequently detected, e.g., to allow for the visualization of the less sensitive detection means. The desired pore rating of the membrane is a function of the size of the biological molecule that is being detected as well as the size of the detecting means (e.g., a radiolabeled oligonucleotide, an antibody, etc.). Preferably, the pore rating of the membrane is in the range of 0.1 μm - 20 μm; more preferably, the pore rating is in the range of 1 μm - 10 μm; even more preferably, the pore rating is in the range of 2 μm - 7 μm; and most preferably, the pore rating is in the range of 3 μm - 6 μm. It should be noted that when a porous membrane is desired, no coating thereof should act to significantly block the pores of the membrane. However, whereas a membrane used within the context of a blotting assay may or may not be porous, depending on considerations such as the sensitivity of the detection means, it is undesirable that such a membrane allow for the lateral diffusion of biological molecules. In contrast, membranes used within the context of immunodiagnostic assays desirably allow for the lateral diffusion of biological molecules.

The membrane also can comprise a hydrophilic compound such that the surface of said membrane is hydrophilic. Like the cellulose compound, the hydrophilic compound can exist as a coating on the

membrane when the ex situ process for membrane formation is utilized; the hydrophilic compound can be in admixture with the polymer solids from which a membrane comprising the admixture is formed by traditional methods (i.e., the in situ process); or alternatively the hydrophilic compound can be both a coating on the membrane and in admixture with the polymer solids of the membrane. For purposes of economy in membrane preparation, it is preferred that, when the ex situ process is utilized to prepare the present inventive membrane, the coating solution comprising the cellulose compound should further comprise the hydrophilic compound. While any suitable hydrophilic compound can be utilized within the context of the present inventive membrane, preferred hydrophilic compounds include surfactants and polymeric wetting agents. Preferably, the surfactant is ionic; more preferably, the surfactant is anionic; even more preferably, the surfactant is a monodentate sulfonate and/or an alpha olefin sulfonate surfactant; and most preferably, the surfactant is Bioterge AS-40, manufactured by Stepan Co. The polymeric wetting agent is preferably a polyquaternary amine, and the preferred polyquaternary amines are those described in U.S. Patent 5,021,160 (Wolpert) as a copolymer of 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and either N-(isobutoxymethyl)acrylamide (IBMA) or 2-hydroxyethyl methacrylate (HEMA).

Furthermore, following formation of the membrane, the membrane can be further modified in any suitable way in accordance with its intended use. In other words, the membrane's immunodiagnostic and/or blotting properties

can be enhanced via modification of the membrane. For example, within the context of an immunodiagnostic assay, the signal-to-noise ratio of the membrane can be enhanced by attaching, to the membrane, any suitable detecting agent (e.g., antibodies which fluoresce upon binding specific antigens). Methods for attaching acceptor molecules (e.g., antibodies) to membranes are well known in the art. *See, generally,* U.S. Patent 4,886,836 (Gsell et al.). In addition, the membrane can be cut into a particular size and/or shape or placed in housings suitable for its intended use.

The membrane can be supported or unsupported. If it is desired that the membrane be supported, any suitable support can be used within the context of the present invention, e.g., a woven or non-woven support.

Methods of Preparation

As previously stated, the present invention provides two methods of preparing membranes: (1) ex situ preparation whereby a coating is applied ex situ to a pre-formed base membrane comprising the polymer solids and (2) in situ preparation whereby the cellulose compound is admixed with the polymer solids and the admixture is used to prepare the membrane.

When utilizing the ex situ preparative method, a membrane is prepared by selecting a pre-formed base membrane comprising the polymer solids and then uniformly coating the base membrane with a coating solution that comprises the cellulose compound and a cellulose dissolving agent.

The cellulose dissolving agent is a solvent with respect to the cellulose compound but is a non-solvent with respect to the selected pre-formed base membrane. By "non-solvent" it is meant that the cellulose dissolving agent is either a poor solvent with respect to the pre-formed base membrane, or, alternatively, a solvent which has absolutely no effect on the performance of the base membrane. A poor solvent is one which may cause the base membrane to swell but not dissolve or one which, under the process conditions (e.g., solvent concentration, time of contact, temperature, etc.), causes no significant or substantial amount of the base membrane to dissolve. The cellulose dissolving agent used within the context of the present invention is chosen so that the performance of the base membrane is not significantly or substantially effected. While any suitable cellulose dissolving agent can be used within the context of the present invention, preferred cellulose dissolving agents include methylacetate and methanol. The concentration of cellulose compound dissolved in the cellulose dissolving agent should be such that, if the membrane to be coated is porous, the cellulose compound will not substantially obscure the pores. Preferably the concentration of cellulose compound in the coating solution is from about 0.1 wt.% to about 5 wt.%; more preferably, from about 0.2 wt.% to about 2 wt.%; and most preferably, from about 0.3 wt.% to about 1 wt.%.

After the base membrane is uniformly coated with the coating solution, the coating is cured by any suitable method, e.g., traditional methods well known in the art. It is important to note that the coating solution can

contain other materials which may enhance the properties of the membrane. For example, the coating solution can contain a hydrophilic compound which renders the resulting membrane hydrophilic, as previously discussed.

When utilizing the in situ preparative method, a membrane is prepared by selecting polymer solids and admixing the polymer solids with the cellulose compound such that the cellulose compound is uniformly distributed throughout the surface of the resulting membrane. After admixing the cellulose compound with the polymer solids, the membrane is formed through any suitable method, e.g., conventional methods which are well known in the art, particularly through a coating process. *See, generally,* U.S. Patent 4,707,266. Of course, the membrane prepared by the in situ preparative method can be utilized as a base membrane in the ex situ preparative method wherein it may undergo coating. Furthermore, the admixture can contain other materials which may enhance the properties of the membrane. For example, the admixture can contain a hydrophilic compound which renders the membrane hydrophilic, as previously discussed.

Methods for forming membranes, whether by virtue of the ex situ or in situ process, are well known in the art. Such methods for membrane formation include but are not limited to irradiative polymerization of unsaturated monomers in a solvent in which the monomer is soluble but the polymer is not, as disclosed in U.S. Patent 4,466,931; graft-polymerization to form a gel followed by shearing to form a thixotropic mixture which may be cast to form a membrane, as disclosed in U.S. Patent 4,374,232; thermally induced precipitation; membrane

coagulation due to solvent leaching, as disclosed in EP 0 036 947; membrane coagulation in a humid atmosphere, as illustrated by U.S. Patents 4,900,499, 4,964,490, and 5,108,607; and membrane casting, as disclosed in U.S. Patents 3,876,738, 4,340,479, 4,473,474, 4,673,504, 4,708,803, and 4,711,793. Other patents and publications which illustrate the preparation of membranes include U.S. Patent 4,629,563, EP 0 036 315, EP 0 037 185, EP 0 165 077, DE 26 51 818, DE 28 29 630, DE 33 27 638, DE 33 42 824, DE 37 01 633, GB 1 295 585, GB 1 473 857, and GB 1 495 887.

In either the ex situ or in situ process, the membrane produced therefrom can be supported on a suitable support, e.g., a woven or non-woven support. For ex situ membranes, the finished membrane can be laminated to a support by any suitable means, e.g., by way of direct thermal lamination or by way of a suitable adhesive. For in situ membranes, the membrane can be cast on the desired support. It is important that if a porous membrane is desired, then the means used to attach the support to the membrane not cause the membrane to significantly lose its porosity.

Both the ex situ and in situ preparative methods can further comprise steps involving the recovery of a membrane and the testing of the immunodiagnostic and/or blotting properties of the recovered membrane such that the preparative method can be adjusted or optimized in response to the test's results in order to alter or enhance the immunodiagnostic and/or blotting properties of the membrane. In other words, the preparative method can include a feedback mechanism whereby the membrane's

immunodiagnostic and/or blotting properties are tested and, in response to the test's results, the process conditions of the preparative method are adjusted so as to yield a membrane with altered or enhanced properties.

As previously stated, optimal immunodiagnostic and/or blotting properties include a membrane's ability to be safely used in a laboratory environment (e.g., the membrane is not flammable or explosive), its ability to be uniformly manufactured in order to yield consistent experimental results, its hydrophilicity, and its ability to strongly bind biological molecules of interest while weakly binding biological molecules not of interest (i.e., the membrane is capable of a high signal-to-noise ratio). Therefore, any membrane prepared by the foregoing preparative methods can be tested for its immunodiagnostic and/or blotting properties, and the process conditions of the preparative method can be adjusted in response to the test so as to enhance or otherwise alter the immunodiagnostic and/or blotting properties of the membrane produced therefrom.

The suitability of the present inventive membranes for any particular immunoassay and/or blotting method, of course, is determined on a case by case basis. Such testing is routine to those skilled in the art and is practiced, for example, for each immunodiagnostic and/or blotting product both prior to commercialization as well as for quality control during production.

Methods of Use

The present inventive membrane can be used within the context of any application where it is desired to

detect a biological molecule of interest. While the membrane can be used in any suitable way, preferably, the method for using the present inventive membrane comprises (a) contacting the membrane with a fluid comprising the biological molecule of interest and (b) detecting the biological molecule of interest on the membrane. Alternatively, the method comprises (a) contacting the membrane with a fluid comprising the biological molecule of interest, (b) allowing the fluid to laterally diffuse through the membrane, and (c) detecting the biological molecule of interest on the membrane.

Immunodiagnostic and Blotting Assay Kits

Another embodiment of the present invention is a kit which can be used for immunodiagnostic and/or blotting assays.

The immunodiagnostic assay kit comprises a membrane and a means for detecting a biological molecule of interest. While any suitable detection means can be utilized within the context of the present invention, the detection means is preferably a dye, an interchelating agent, a fluorescent probe, or a radioactive probe.

The blotting assay kit comprises a membrane and a blotting solution. While the blotting solution can be any solution utilized within the context of a blotting assay, the blotting solution is preferably a pH-buffering solution or a solution with a specific ionic concentration.

EXAMPLES

The following examples further illustrate the present invention but, of course, should not be construed as in any way limiting its scope.

In Examples 1-4, a commercially available, hydrophilic, polyethersulfone, microporous membrane having a nominal pore size of 5 μm, available from Gelman Sciences, Inc., as Supor® 5000, was used to prepare several lateral flow microporous membranes suitable for use in immunoassays. The membrane, in each case, was used dry.

Example 1

A coating solution of nitrocellulose was prepared at room temperature. Hercules RS and SS nitrocellulose resins (5 g) having degrees of nitration of 12% and 11%, respectively, were mixed with methylacetate (995 g) to yield a coating solution with an average degree of nitration between 11.3% and 11.8%. The mixture was agitated for 4 hours, and a clear solution was obtained. Samples of dry, hydrophilic, polyethersulfone membrane with a 5 μm nominal pore rating were dipped into the coating solution and allowed to dry at room temperature. The membranes were stored at room temperature for use in Example 3.

Example 2

A coating solution of nitrocellulose (5 g) and methanol (995 g) was prepared at room temperature. The mixture was agitated for 4 hours, and a clear solution was obtained. Samples of dry, hydrophilic, polyethersulfone membrane with a 5 μm nominal pore rating

were dipped into the coating solution and allowed to dry at room temperature. The membranes were stored at room temperature for use in Example 3.

Example 3

Bioterge AS-40 (1 g) (available from Stepan Co.) was mixed with deionized water (999 g), and the mixture was agitated for 4 hours. A clear, slightly yellow solution was obtained. Samples of the membranes prepared in Examples 1 and 2 were dipped into the solution and dried at 60 °F (15.6 °C) for 1 hour. The membranes were hydrophilic after treatment and were stored at room temperature as suitable for use in immunodiagnostic assays.

Example 4

A solution of Bioterge AS-40 (1 g), nitrocellulose (5 g), and methanol (994 g) was prepared. Samples of dry, hydrophilic, polyethersulfone membranes with a 5 μm nominal pore rating were dipped into the solution and allowed to dry at room temperature. The membranes were stored at room temperature and were suitable for use in immunodiagnostic assays.

Example 5

This example illustrates in situ membrane formation. An admixture was prepared by dissolving 6.8 wt.% polyethersulfone polymer in 9.4 wt.% dimethylformamide, 65.8 wt.% polyethylene glycol 400, 0.4 wt.% polyvinylpyrollidone K90, 9.8 wt.% N-methylpyrollidone, and 1.6 wt.% Aquazol 500 (available from Polymer Chemistry Innovations), to which was added 0.2 wt.% nitrocellulose

and 0.2 wt.% Bioterge AS-40. The balance of the admixture was water. The admixture was applied to a glass plate at a thickness of about 18 mil (457.2 μm) and placed in a low air velocity humid environment until the composition became cloudy. The resulting membrane was then dried in air to form a membrane with a 5 μm nominal pore rating. The membrane was dipped in surfactant and dried to form a hydrophilic membrane suitable for immunoassay use.

Example 6

A microporous lateral flow membrane as prepared in Example 4 was compared to a commercial nitrocellulose membrane of similar pore size (available from Millipore Corp., Bedford, MA). The test protocol utilized rabbit IgG antigen, mouse anti-rabbit IgG conjugate, mouse anti-rabbit IgG (γ-specific) as a capture line, and goat anti-mouse IgG as a control line, as previously indicated.

In the test protocol utilized in the present example, colloidal gold conjugates, such as those disclosed in EP 0 250 137 were utilized. The gold colloids were prepared by adding 4 ml of 1% gold chloride to 200 ml boiling water to which was added 12 ml of 1% trisodium citrate. The solution was mixed well. The gold colloid thus produced contained 30-40 nm gold beads which displayed a wine-red color. The colloid can be stored in the dark at room temperature.

To prepare the gold conjugate, 1 ml of colloidal gold was added to a microcentrifuge tube, and 25 μl of 1 mg/ml dialyzed mouse anti-rabbit IgG (Pierce 31213) and 100 μl of 20 mM borax solution were added and left to stand for 1

hour at room temperature. Next, 100 µl of 1% BSA/20 mM borax solution is added to the tube, at which time the tube was centrifuged at 15k for 50 minutes. The supernatant was withdrawn, and the pellet was resuspended in 1 ml of 0.1% BSA/2 mM borax buffer. The suspension was again centrifuged, and the obtained pellet was resuspended in 250 µl of the buffer solution. The gold-conjugated antibody was stored at 4 °C prior to use.

The gold conjugated antibody was diluted 1:4 with 100 mM Tris pH=7 containing 10% BSA, 0.1% Tween 20, and 10% sucrose, and applied to a glass conjugate pad by dipping, followed by drying at 58 °C for 30 minutes. The membranes to be tested were sprayed with the appropriate solutions with the control line and test line set at 0.5 mm line width and sprayed twice at a loading of 0.2 µl/cm. The assembled strip constituted, in linear order, a sample application pad, a conjugate pad with mouse anti-rabbit IgG conjugate, a membrane having both a test line (mouse anti-rabbit IgG (γ-specific)/1 mg/ml) and a control line (goat anti-mouse IgG (2 mg/ml), and an absorption pad.

Samples of 200 µl of rabbit IgG antigen were applied to the sample application pad and allowed to migrate through the conjugate pad and into the membrane. The antigen was detected at the test line of the membrane. The results are presented in the table below:

Membrane	Reaction Time (Minutes)	Sensitivity	Signal Appearance
Present Invention	2.5	50 ng/ml	Good
Comparative	3.0	50 ng/ml	Good

The results indicate that the present inventive membrane exhibited a faster response time than the nitrocellulose-based membrane while being equivalent in sensitivity and signal appearance. In addition, the present inventive membrane was far stronger and far less flammable.

All of the references cited herein, including patents, patent applications, and publications, are hereby incorporated in their entireties by reference.

While this invention has been described with an emphasis upon preferred embodiments, it will be obvious to those of ordinary skill in the art that variations of the preferred embodiments may be used and that it is intended that the invention may be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications encompassed within the spirit and scope of the invention as defined by the following claims.

WHAT IS CLAIMED IS:

1. A membrane comprising (a) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (b) a cellulose compound which allows for the detection of a biological molecule of interest, wherein said cellulose compound is uniformly distributed throughout the surface of said membrane.

2. The membrane of claim 1, wherein said polymer solids comprise 80 wt.% or more of a fully synthetic organic polymer and optionally about 20 wt.% or less of a polymer of natural origin.

3. The membrane of claim 2, wherein said cellulose compound is nitrocellulose.

4. The membrane of claim 3, wherein said membrane has a pore rating of about 0.1-20 μm.

5. The membrane of claim 3 or 4, wherein said nitrocellulose is a coating on the surface of said membrane.

6. The membrane of claim 3 or 4, wherein said nitrocellulose is in admixture with said polymer solids.

7. The membrane of any of claims 1-6, further comprising a hydrophilic compound such that the surface of said membrane is hydrophilic.

8. The membrane of claim 7, wherein said hydrophilic compound is a surfactant.

9. The membrane of claim 1, wherein said cellulose compound is a blend of nitrocellulose and a cellulose derivative selected from the group consisting of an ether derivative of cellulose, an ester derivative of cellulose, a xanthate derivative of cellulose, and combinations thereof.

10. The membrane of claim 9, wherein said ether derivative of cellulose is selected from the group consisting of methylcellulose, carboxylated alkyl cellulose, and hydroxyalkylcellulose, said ester derivative of cellulose is cellulose acetate, and said xanthate derivative of cellulose is selected from the group consisting of rayon, cellophane, and viscose.

11. The membrane of claim 10, wherein said hydroxyalkylcellulose is hydroxypropylcellulose.

12. The membrane of claim 9, wherein said cellulose compound is a coating on the surface of said membrane.

13. The membrane of claim 9, wherein said fully synthetic organic polymer is selected from the group consisting of polyethersulfone, polysulfone, polyamide, polyolefin, polyimide, halogenated polyolefins, and combinations thereof.

14. The membrane of any of claims 9-13, further comprising a hydrophilic compound such that the surface of said membrane is hydrophilic.

15. The membrane of any of claims 1,2, and 9-14, wherein said cellulose compound is in admixture with said polymer solids.

16. The membrane of any of claims 1, 2, and 9-14, wherein said cellulose compound is a coating on the surface of said membrane and in admixture with said polymer solids.

17. A membrane comprising (a) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (b) a cellulose compound, wherein said cellulose compound is in admixture with said polymer solids.

18. A method of preparing a membrane comprising (a) selecting a base membrane comprising polymer solids, said polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin, and (b) uniformly coating said base membrane with a coating solution, said coating solution comprising a cellulose compound which allows for the detection of a biological molecule of interest and a cellulose dissolving agent which is a solvent for said cellulose compound and is a non-solvent for said base membrane, to provide a membrane wherein

said cellulose compound is uniformly distributed throughout the surface of said membrane.

19. The method of claim 18, wherein said polymer solids comprise 80 wt.% or more of a fully synthetic organic polymer and optionally about 20 wt.% or less of a polymer of natural origin.

20. The method of claim 19, wherein said cellulose compound is nitrocellulose.

21. The method of claim 20, wherein said membrane has a pore rating of about 0.1-20 μm.

22. The method of any of claims 18-21, wherein said coating solution further comprises a hydrophilic compound such that the surface of said membrane is hydrophilic.

23. The method of claim 18, wherein said cellulose compound is a blend of nitrocellulose and a cellulose derivative selected from the group consisting of an ether derivative of cellulose, an ester derivative of cellulose, a xanthate derivative of cellulose, and combinations thereof.

24. The method of claim 23, wherein said ether derivative of cellulose is selected from the group consisting of methylcellulose, carboxylated alkyl cellulose, and hydroxyalkylcellulose, said ester derivative of cellulose is cellulose acetate, and said

xanthate derivative of cellulose is selected from the group consisting of rayon, cellophane, and viscose.

25. The method of claim 24, wherein said hydroxyalkylcellulose is hydroxypropylcellulose.

26. The method of claim 23, wherein said fully synthetic organic polymer is selected from the group consisting of polyethersulfone, polysulfone, polyamide, polyolefin, polyimide, polyvinylidenefluoride, and combinations thereof.

27. The method of any of claims 23-26, wherein said coating solution further comprises a hydrophilic compound such that the surface of said membrane is hydrophilic.

28. A method of preparing a membrane comprising (a) admixing (i) polymer solids comprising 60 wt.% or more of a fully synthetic organic polymer and optionally about 40 wt.% or less of a polymer of natural origin and (ii) a cellulose compound, and (b) casting the admixture of step (a) to prepare a membrane wherein said cellulose compound is uniformly distributed throughout the surface of said membrane.

29. The method of claim 28, wherein said polymer solids comprise 80 wt.% or more of a fully synthetic organic polymer and optionally about 20 wt.% or less of a polymer of natural origin.

30. The method of claim 29, wherein said cellulose compound is nitrocellulose.

31. The method of claim 30, wherein said membrane has a pore rating of about 0.1-20 μm.

32. The method of any of claims 28-31, wherein the surface of said membrane is hydrophilic.

33. The method of claim 28, wherein said cellulose compound is selected from the group consisting of nitrocellulose, an ether derivative of cellulose, an ester derivative of cellulose, a xanthate derivative of cellulose, and combinations thereof.

34. The method of claim 33, wherein said ether derivative of cellulose is selected from the group consisting of methylcellulose, carboxylated alkyl cellulose, and hydroxyalkylcellulose, said ester derivative of cellulose is cellulose acetate, and said xanthate derivative of cellulose is selected from the group consisting of rayon, cellophane, and viscose.

35. The method of claim 34, wherein said hydroxyalkylcellulose is hydroxypropylcellulose.

36. The method of claim 28, wherein said fully synthetic organic polymer is selected from the group consisting of polyethersulfone, polysulfone, polyamide, polyolefin, polyimide, polyvinylidenefluoride, and combinations thereof.

37. The method of any of claims 33-36, wherein the surface of said membrane is hydrophilic.

38. A method of using the membrane of any of claims 1-17 to detect a biological molecule of interest comprising (a) contacting said membrane with a fluid comprising said biological molecule of interest and (b) detecting said biological molecule of interest on said membrane.

39. An immunodiagnostic assay kit comprising the membrane of any of claims 1-17 and a means for detecting a biological molecule of interest.

40. A blotting assay kit comprising the membrane of any of claims 1-17 and a blotting solution.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/20475

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/545 B01D67/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 964 990 A (KRAUS MENAHAM ET AL) 23 October 1990 cited in the application see the whole document ---	1
X	EP 0 402 196 A (TERUMO CORP) 12 December 1990 see example 1 ---	1,2,4,7, 8,14-16, 28,29, 31-34
X	GB 2 081 604 A (CELANESE CORP) 24 February 1982 see claims 1-3 --- -/--	1,2,4,7, 8,14-16, 28,29, 31-34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 February 1998	27/02/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Wells, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte. ional Application No
PCT/US 97/20475

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category ³	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 280 560 A (EASTMAN KODAK CO) 31 August 1988 see example 1	1,2,4,7, 14-19, 21,22, 28,29, 31-33, 37-40
X	EP 0 257 635 A (DANSKE SUKKERFAB) 2 March 1988 see examples 11,12,17	1,2,4,7, 8,14-19, 21,22, 28,29, 31-34
X	EP 0 272 842 A (PALL CORP) 29 June 1988 see example 1	1,2,4,7, 14-19, 21,22, 28,29, 31-34
A	US 4 601 828 A (GERSHONI JONATHAN M) 22 July 1986 see example 1	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/20475

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4964990 A	23-10-90	US 4900449 A	13-02-90
		US 5108607 A	28-04-92
EP 0402196 A	12-12-90	JP 3008421 A	16-01-91
		DE 69021452 D	14-09-95
		DE 69021452 T	01-02-96
		US 5120440 A	09-06-92
GB 2081604 A	24-02-82	US 4359510 A	16-11-82
		CA 1169118 A	12-06-84
		DE 3130069 A	04-03-82
		FR 2488051 A	05-02-82
		JP 1904850 C	08-02-95
		JP 6019980 B	16-03-94
		JP 57055064 A	01-04-82
		US 4438185 A	20-03-84
EP 0280560 A	31-08-88	US 4847199 A	11-07-89
		US 4828980 A	09-05-89
		CA 1308349 A	06-10-92
		DE 3850981 D	15-09-94
		DE 3850981 T	30-03-95
		DE 3882058 A	05-08-93
		DE 3882058 T	13-01-94
		DE 3883808 D	14-10-93
		DE 3883808 T	28-04-94
		DE 3884235 D	28-10-93
		DE 3884235 T	28-04-94
		DE 3884979 D	25-11-93
		DE 3884979 T	19-05-94
		DE 3889479 D	16-06-94
		DE 3889479 T	22-12-94
		EP 0280556 A	31-08-88
		EP 0280557 A	31-08-88
		EP 0281327 A	07-09-88
		EP 0280559 A	31-08-88
		EP 0280561 A	31-08-88
		JP 63229366 A	26-09-88
		JP 1972401 C	27-09-95
		JP 6064061 B	22-08-94

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/20475

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0280560 A		JP 63229367 A	26-09-88
		JP 63243758 A	11-10-88
		JP 1895410 C	26-12-94
		JP 6014047 B	23-02-94
		JP 63231265 A	27-09-88
		JP 63289454 A	25-11-88
		JP 63231268 A	27-09-88
EP 0257635 A	02-03-88	DK 169616 B	27-12-94
		DE 3784410 A	08-04-93
		ES 2053488 T	01-08-94
		IE 60368 B	13-07-94
		JP 2677360 B	17-11-97
		JP 63068646 A	28-03-88
		US 5019261 A	28-05-91
		US 5091086 A	25-02-92
EP 0272842 A	29-06-88	US 4964989 A	23-10-90
		CA 1312508 A	12-01-93
		DE 3779412 A	02-07-92
		GB 2198972 A,B	29-06-88
		JP 63168561 A	12-07-88
		KR 9600656 B	11-01-96
		US 4968533 A	06-11-90
US 4601828 A	22-07-86	US 4512896 A	23-04-85
		AU 577276 B	22-09-88
		AU 2497884 A	30-08-84
		CA 1207737 A	15-07-86
		DE 3486231 D	25-11-93
		DE 3486231 T	14-04-94
		DE 3486313 D	07-07-94
		DE 3486313 T	10-11-94
		EP 0138841 A	02-05-85
		EP 0343387 A	29-11-89
		FR 2540629 A	10-08-84
		JP 3076868 B	06-12-91
		JP 60501126 T	18-07-85
		WO 8403055 A	16-08-84

Inventor: **Bormann** Application Number: **09/806322** Date: **6/5/01; pd 10/2/98**

Cl. #	Dep. on	Limitation	Kraus 7/98 US 5,783,094	Onodera 4/95 US 5,406,581	
1	--	A filter comprising	Abs	Abs	
		Housing with inlet, outlet and flow path in between	Ex 24	Abs	
		Filter in housing, comprising	Ex 24	Abs	
		First: Porous fibrous leukocyte depletion medium	3(40-63)	4(12-23)	
		with CWST 70 dynes/cm	--	17(38-68)	
		Second: Porous membrane 5 microns or less pore size, down – stream of first	3(40-63)	[Col 20] [no first and 2nd]	
		Plasma pass thru, leukocyte filtered.	Working examples	Working examples 21-23	
2	--	Same as 1 except: first also red cell barrier	--	--col 20 obv	
3	1	Same as 2 (duplicate)	XX	Xx	
4	1	First element comprises meltblown	--	15(54-63)	
5	1	First element at least two layers	Working examples	Ex 21	
6	1	First has CWST at least 90	--	17(53-68)	
7	1	Includes no more than one membrane	3(40-46)	4(23-48)	
8	1	Filter arranged to substantially prevent RBC thru	--	Obv Col 20	
9	1	System for processing..comprising..device in claim 1 and	--	65(29-48)	
		First and second container suitable for blood, device between them	--	Do	
10	--	Method for processing …comprising..	Working ex	Ex 21	
		Passing plasma thru filter device comprising	Do	Do	
		Filter including fibrous leuko depletion medium and membrane	Working examples	--	
		Collecting filtered plasma free of luk	Do	Do	
11	--	Same as 10 except .. rbc barrier	--	4(24-48)	

		instead of luk depletion			
12	--	Method comprising..	Yes		
		Processing a biological fluid with supernatent layer comprising plasma-luk; sediment layer with RBC	Working examples	Ex 21	
		Passing luk rich thru filter having luk-depletion fiber medium and mmb	Do	Do	
		Collecting filtered plasma rich fluid free of RBC and luk	--	Do	
13	1 (12)	METHOD of claim 1 where ...fluid comprises luk and platelet depleted bio fluid	--	-- Obv	
14	--	Method comprising	Yes		
		Depleting luk and platelet from rbc containing fluid	--	Do	
		Processing ... depleted .. to supernatent ... and sediment ...	--	Do	
		Supernatent thru filter – depletes luk and prevents rbc..	--	Do (no mmb – obv)	
		Collecting rbc and luk free plasma	--	--	
17	1	2nd ..mmb..comprises...pore size 0.3-3 mic	3(47-52)	Col 20	
18	7	Duplicate of 17	XX	Xx	
19	3	2nd .. 0.3 – 3 mic	3(47-52)	Col 20	
20	--	Similar to 1	XX	Xx	